APPENDIX W

PROTOCOL FOR PREPARATION AND HANDLING

REFERENCE MATERIALS

Changes made to this protocol;

1. **Major rewriting was done to reflect the fact that reference material will not be vacuumed into the liner as part of preparation.**

2. **Only one reference material per batch will be used in the preparation of environmental material.**

3. **The new NIST SRM for blood has been described in this section.**

4. **SRM will not be used for the Bioavailable Pb method.**

PROTOCOL FOR PREPARATION AND HANDLING

REFERENCE MATERIALS

1.0 PREPARATION OF REFERENCE MATERIALS

1.1 Three reference material types will be used for this project, all certified and manufactured by NIST. The Standard Reference Materials (SRM) will be used to verify instrument calibration before sample analysis proceeds. Other samples used will be the monthly Blood Lead Proficiency samples from the State Laboratory at the University of Wisconsin. The primary human blood standards, which are used for ASV calibration at the Kennedy Institute, which have been certified by Isotope Dilution Mass Spectrometry may also be used.

1.2 SRM#2704, Buffalo River Sediment, will be used for verification of dust and soil sample analysis. The material will be dried at 110°C in a convection oven before use. The lot number and certification will be stored in the laboratory for each bottle ordered. The material will be stored according to the manufacturers recommendations.

1.3 SRM#955a, Lead in Blood, will be used for verification of blood lead analysis by Graphite Furnace. The SRM which consists of 4 x 2 mL vials, each with a different concentration of Pb;

955a-1	5.01 ± 0.09 µg/dL
955a-2	13.35 ± 0.13 µg/dL
955a-3	30.63 ± 0.32 µg/dL
955a-4	54.43 ± 0.38 µg/dL

A 1:10 dilution of the sample, with matrix modifier, will be made before analysis.

1.4 SRM#1643c, Trace Metals in Water, will be used to verify the calibration of the Graphite Furnace for the analysis of drinking water samples.

SRM#1643c 35.3 ng/mL

1.5 All reference materials will be handled according to the manufacturers instructions. Blood will be stored at -10 °C in a freezer.

1.6 Reference materials will not be used beyond the expiration dates on the containers.

1.7 Reference material #2704, Buffalo River Sediment, may be used to monitor the performance of the modified HVS3 cyclone sampler. At the beginning of each sampling campaign the sampling teams will use each cyclone to sample 10 x 0.500 g samples of SRM#2704. The results will be compared to the original data from the cyclone test to monitor the performance of both the vacuums and the cyclone sampler. Four modified HVS3 cyclones will be used for this test. A record of the performance of each cyclone will be retained in the laboratory.

2.0 SOIL AND DUST

2.1 SRM#2704 will be dried in the oven at 110°C for 1 hour and cooled in a desiccator before use for soil and dust samples.

2.2 The dried material will be subsampled for inclusion in sample batch preparation during processing.

2.3 At the time of sample preparation the SRM will be assigned a unique barcode label with the first letter of the matrix type as an indicator of its origin. For dust samples the SRM will be Dnnnnn (e.g. D00010). This will ensure that there is no distinction made between regular samples and Quality Control material and the material is presented blind to the analyst.

2.4 For dust samples the SRM will be treated the same as the regular samples. The SRM will be dried overnight, cooled and reweighed with the sample batch.

2.5 For soil samples the SRM will not be sieved and homogenized but will be included after this step.

2.6 SRM weights will be varied to reflect the maximum weight of the sample batch for dust samples.

2.7 One SRM sample will be prepared for each batch of field samples digested.

3.0 WATER

3.1 25 mL of SRM#1643c will be added to a digestion liner during preparation of water sample batches. The volume will be made up with deionized water (20 mL) and the sample will be treated in the same way as regular samples.

3.2 The recommended volume of SRM#1643c may be changed from time to time as required by the QC Officer.

3.3 The SRM material will receive a unique barcode number during sample batch preparation and will be presented blind to the analyst.

3.4 One SRM will be prepared for each batch of water samples digested.

APPENDIX X

PROTOCOL

FOR GLASSWARE/PLASTICWARE PREPARATION

Changes made to this protocol;

1. **Acid baths will be changed every week; previously every 2 weeks.**

PROTOCOL FOR

GLASSWARE/PLASTICWARE PREPARATION

1.0 SUMMARY OF METHOD

1.1 This procedure is used to prepare lab glassware and plasticware in the Kennedy Krieger Institute Trace Metals Laboratory.

1.2 Teflon^{R} liners used in microwave digestion may use an alternative method of microwave heating to clean vessels. (see 5.11)

1.3 Items which can be exempted from this procedure include those items which have already been screened for positive contributions of lead and found to be free from lead contamination.

1.4 Protective clothing such as gloves and laboratory coats should be worn at all times when handling acids and potentially hazardous chemicals.

2.0 APPARATUS AND MATERIALS

2.1 Glassware Tubs; labelled **"FOR CLEAN GLASSWARE ONLY"**

2.2 Glassware Tubs; labelled **"FOR DIRTY GLASSWARE ONLY"**

2.3 Plastic brushes; assorted sizes.

2.4 Gloves; heavy duty and disposable, powderless vinyl.

2.5 Pipette washers (optional).

3.0 REAGENTS

3.1 Type I Water: minimum resistance 16.67 megaohm-cm, or equivalent.

3.2 Acationox detergent.

3.3 Acid baths: 10%(v/v) HNO_3.

3.4 Acetone.

4.0 QUALITY CONTROL

4.1 Acid baths will be changed every week usually at the beginning of a working week.

4.2 On a weekly basis washing procedures will be checked by the analyst. Three items will be randomly selected and rinsed with a few mL of 10% HNO_3. The absorbance of the rinsate should not be more than 0.005 A-s when run with phosphate matrix modifier on the GFAA. If absorbance of any sample is greater that of the calibration blanks (0.005 A-s) then the washing procedure should be revised and changed accordingly.

4.3 The method blanks prepared during digestion of routine samples will be used to monitor the cleaning process.

4.4 If methods blanks suggest a systematic problem with contamination, then the acid baths will be changed immediately and the cleaning process monitored daily until such time as the problem has been rectified.

5.0 PROCEDURE

5.1 Remove all labels and marks from the glassware and plasticware. Use acetone and a kimwipe to remove indelible pen markings.

5.2 Soak the glassware and plasticware overnight in acationox.

5.3 Scrub the containers inside and out with a brush.

5.4 Rinse the vessels with deionized water six or seven times.

5.5 Soak the vessels in 10% nitric acid overnight.

5.6 Remove the vessels from the acid bath and rinse three or four times with deionized water.

5.7 Allow the dishes to air dry in draining racks.

5.8 Store the vessels in clean labelled plastic bags until used.

5.9 For pipettes use the same procedure without the brushes.

5.10 For rinsing pipettes an automatic rinsing syphon may be used.

5.11 For the cleaning of 100 mL Teflon[r] liners used in sampling and digestion the microwave oven can be used. The liners should be soaked for a few hours in acationox detergent; cleaned with a brush and rinsed with deionized water. See Appendix R for details of how to operate the microwave oven and read the Operators Manual.

5.12 The liners are then filled with 10 mL of 10 % HNO_3 and sealed in vessels as for a normal digestion. The program called EPA WASH is then recalled and run. This heats the vessels at 80% power for 10 mins.

5.13 After cooling the liners are opened and rinsed with deionized water. The liners are then air dried and stored in labelled plastic bags.

5.14 Polypropelyne tubes used in the analysis of samples by ASV are soaked for four hours in acationox before being soaked overnight in 10% HNO_3. The tubes are placed in plastic bags until needed for analysis.

CONTAMINATION CHECK FOR ACID BATHS

DATE	ANALYST	ABSORBANCE A-s	ITEM	DATE GLASSWARE REWASHED IF REQUIRED

APPENDIX Y

REVISED TRACEABILITY FORMS

Kennedy Krieger Institute Repair and Maintenance Study
FIELD SAMPLE TRACEABILITY RECORD

DWELLING ID - ROUND ☐☐☐-☐☐

(Round: IN PI 02 06 12 18 24)

DATE ☐☐-☐☐-☐☐

m m d d y y

Place Bar Code of Field Voided Sample Here	Place Bar Code of Field Voided Sample Here	Place Bar Code of Field Voided Sample Here	Place Bar Code of Field Voided Sample Here

Comments:

(Signatures:) Relinquished by: Transferred to: Date:

11/16/92

Kennedy Krieger Institute Repair and Maintenance Study
LOG-IN TRACEABILITY FORM

Dwelling ID #: _____ **Study Group:** _____ **(Modern, R&M I, II, or III, Abated)**

Sampling Campaign	Date Received (initialed)	Number Received				Comment	Team Leader (name)	Sampling Complete (yes/no)
		DV[1] (n)	S[2] (n)	W[3] (n)	DW[4] (n)			
Pre-R&M/Enroll								
Post (R&M only)								
2-Month (R&M only)								
6 Months								
12 Months								
18 Months								
24 Months								

[1]DV = Dust/Vacuum
[2]S = Soil
[3]W = Water
[4]DW = Dust Wipe

Supervisor: _____ Date: _____

11/16/92

APPENDIX AA

PROTOCOL FOR BLOOD LEAD DETERMINATION

USING GRAPHITE FURNACE WITH ZEEMAN BACKGROUND CORRECTION

1.0 INTRODUCTION

1.1 Lead in whole blood samples is measured by atomic absorption spectrometry based on the method described by Millar et al. The lead is quantified by measurement of light absorbed at 283.3 nm by ground state atoms of lead from a hollow cathode lead lamp. Blood samples, blood based quality control materials, and aqueous standards are diluted 1:10 with a matrix modifier solution containing nitric acid, Triton X-100 and ammonium dihydrogen phosphate. The lead content is determined using an atomic absorption spectrometer equipped with Zeeman background correction. Lead contamination must be carefully avoided throughout all procedures.

1.2 This method has been developed for routine analysis of blood specimens received from childhood lead screening programs. It is based on methods currently in use by Center for Disease Control and New York State Department of Health laboratories.

2.0 MATERIALS AND REAGENTS

2.1 Nitric acid (HNO_3); ULTREX^r II Ultrapure Reagent.

2.2 ASTM Type I water; minimum resistance of 16.67 megohm-cm.

2.3 Triton^r X-100 (alkylaryl polyether alcohol), Baker analyzed.

2.4 Atomic Spectroscopy Standard; Perkin Elmer, 1,000 µg/Ml Pb or equivalent.

2.5 NIST Standard Reference Material 955a; Lead in Blood, with high and low values. Certified by Isotope Dilution Mass Spectroscopy. These will be used as quality control samples.

2.6 Primary blood standard; KKI has human blood lead standards which have been analyzed by IDMS. These may be used as quality control samples.

2.7 Ammonium dihydrogen phosphate; ultrapure; Johnson Matthey.

2.8 Disposable Falcon tubes with caps: Cat. No. 14-956-1D available from Fischer Scientific.

2.6 Disposable polystyrene autosampler cups; 1.5 mL capacity. Perkin Elmer B011-9079.

2.7 Plastic bottles for storing standards; five 125 mL, acid washed.

2.8 Argon gas, zero grade, equipped with an approved gas regulator.

2.9 Pyrolytic coated graphite tubes with preinserted L'vov platform available from Perkin Elmer.

3.0 APPARATUS

3.1 Perkin Elmer Zeeman 5100 Atomic Absorption Spectrometer with AS-60 Autosampler and Decstation 316 SX computer and Epson LQ-850 printer.

3.2 Micromedic Digiflex TX Automatic Pipettor manufactured by ICN Biomedicals, Inc. with an accuracy of ± 1%.

3.3 Vortexer; VWR Model K-550-G.

3.4 Micropippettes (1000, 200, and 20 µL) and disposable tips: Eppendorf or similar. Disposable tips should be trace metal certified.

3.5 Class A volumetric flasks; 1000, 100 and 50 mL capacity, acid washed.

3.6 Thermolyne Speci-mix; model number M26125.

3.7 Volumetric pipettes; 10 mL.

4.0 PREPARATION OF STANDARD SOLUTIONS AND MATRIX MODIFIER

4.1 Stock 10% (v/v) Triton X-100; Using a volumetric pipette, transfer 10 mL of Triton X-100 to approximately 80 mL of deionized water, slightly warmed. Mix thoroughly on a magnetic stirrer for 1 hour. Make up to 100 mL.

4.2 Stock 20% (w/v) $NH_4H_2PO_4$; Make up a 20% solution of ammonium dihydrogen phosphate by adding 20 g to 100 mL of deionized water. Mix, label and date the matrix modifier solution. The solution is stable for about 6 months if kept at 4°C.

4.3 Working solution of matrix modifier; Add 300 mL of deionized water to a 500 mL flask. Using a micropipette, carefully add 1.00 mL of conc. HNO_3 and swirl to mix. Add 25 mL of 10 % Triton X-100, 5 mL of 20% (w/v) $NH_4H_2PO_4$ and make up to 500 mL with deionized water. Mix thoroughly, label and date. Working solution should be prepared weekly. The solution contains 0.2 % HNO_3, 0.5% Triton X-100 and 0.2% (w/v) ammonium dihydrogen phosphate.

5.0 PREPARATION OF STANDARDS

5.1 Intermediate concentrations of Pb standard solutions are prepared by serial dilution of 1000 mg/L stock, keeping a nitric acid concentration of 1% (v/v). A 10x concentrated solution is made for each of the five standards.

5.2 Solution A: 10 mg/L intermediate Pb stock solution; Pipet 10 mL of 1000 mg/L Pb stock into a 1000 mL volumetric flask. Add 10 mL of conc. HNO_3 and dilute to the mark to obtain 1mg/L Pb in 1% HNO_3. Prepare monthly.

5.3 Solution B: 1 mg/L intermediate Pb stock solution. Pipet 10 mL of 10 mg/L (solution A) into a 100 mL volumetric flask. Add 1 mL of conc HNO_3 and dilute to the mark to obtain a 1 mg/L Pb solution in 1% HNO_3. Prepare monthly.

5.4 Weekly stock solutions;

Pipet 5 mL of 1 mg/L (B) into a 100 mL volumetric flask and dilute to volume with 1% HNO_3. The final concentration of the solution is 50 µg/L. Use the following table as a guide in preparing the other standards in 100 mL volumetric flasks.

Stock Used mg/L Pb	Volume Used mL	Final Conc. µg/L Pb
1	5	50
1	10	100
10	2	200
10	3	300
10	4	400
10	5	500

5.5 Working Standards (S1-S5); Dilute daily from weekly stock solutions; 100 µL of weekly stock + 900 µL of matrix modifier solution in an autosampler cup.

6.0 PREPARATION OF BLOOD SPECIMENS AND QUALITY CONTROL SAMPLES

6.1 Always use protective gloves when handling human blood.

6.2 Set the sampling pump at 100 µL and the dispensing pump at 300 µL. Place the dispensing tube in the matrix modifier solution and flush the system a few times by pressing the foot pedal.

6.3 Use the foot pump to dispense 100 µL of the well mixed blood sample into the dispensing tube. Wipe the excess off the outside of the pipette with a kimwipe.

6.4 Dispense the sample with 300 µL of matrix modifier solution into a falcon tube. Touch the last drop to the side of the vessel without actually touching the vessel with the tip of the pipette.

6.5 Pick up 100 µL of air and 300 µL of matrix modifier solution and wipe excess with a Kimwipe. Dispense into the same tube.

6.6 Repeat the procedure once more and mix gently on the vortexer.

6.7 A total of 100 µL of specimen blood and 900 µL of matrix modifier solution have been dispensed making a 1:10 dilution of the specimen blood. The total volume of diluted blood is 1000 µL.

6.8 Prepare two quality control samples for each analytical run using the procedure above. A high and a low Pb concentration are required.

7.0 INSTRUMENT SETUP

7.1 Refer to the Reference Manual and Users Manual for the 5100 Zeeman furnace setup.

7.2 Turn on Zeeman furnace, AAS, HGA 600 printer and DEC 316sx computer in that order.

7.3 Turn on the argon gas and verify that tank pressure is > 100 psi. The outlet pressure should be 40-50 psi.

7.4 Before running blood samples install a new tube and condition it using the program for conditioning graphite tubes. Clean the furnace before inserting the new tube.

7.5 Select the File called Blood_Pb.GEL from the element files.

7.6 Check the autosampler reservoir and waste container. If necessary empty the latter and fill the former with 100 µL of 10 % Triton X-100 dissolved in 2 L of deionized water. Fill the blank container at position 0 with fresh matrix modifier.

7.7 Examine the tip of the capillary injection tubing and if it is bent or discolored, prepare a fresh piece by pulling it out with a pliers and cutting at a 45° angle. Check the alignment and the penetration into the graphite tube. Adjust if necessary.

7.8 A sample/standard injection of 12 µL will be used.

8.0 INSTRUMENT OPERATION

8.1 In the AS-60 Control window, click on the box marked RUN STANDARDS. The software will proceed with running the blank

and each standard in duplicate. If the blanks absorbance is > 0.005 then stop the run and call the supervisor.

8.2 While the standard are running prepare the ID/WT file. Use the bar code reader or enter manually the sample ID.

8.3 When the standards have finished running the correlation coefficient should be > 0.999 else the run should not proceed. Remake the standards if necessary.

9.0 QUALITY CONTROL

9.1 After calibration the NIST low and high samples should be run and the results should be ± 2 µg/dL of the true or certified value.

9.2 All samples will be prepared in duplicate and duplicate readings will be within 1 µg/dL of each other otherwise the samples will be reprepared and rerun.

9.3 The low and high blood quality control standards will be run after every 8 samples (four duplicate pairs) and the run will not be valid unless the measured value is ± 2 µg/dL of the true or certified value.

9.4 As required other quality control samples will be run in order to establish the proficiency the laboratory method. These samples will be run on a monthly basis and will be provided by Wisconsin State Laboratory. Other proficiency samples may also be run as required.

10.0 CALCULATIONS

10.1 The characteristic mass or absolute sensitivity is defined as the mass of an element (pg) required to produce an absorbance of 1% (0.0044 Abs-sec).

$$\text{Characteristic Mass} = \frac{\text{conc. } (\mu g/L) \times 0.0044 \times \text{VOL}}{\text{abs-sec}}$$

The established characteristic mass for lead is 12 pg. However it has been shown that gas flow of about 30 mL/min during atomization markedly improves precision at the cost of

decreased sensitivity. The decrease in precision will not compromise the detection limit for the method. This option may be used in the event of poor precision.

10.2 Precision is defined in terms of the relative standard deviation (RSD) or coefficient of variation, and is expressed as a percentage;

$$RSD = \frac{\sigma_{(n-1)}}{\text{mean}} \times 100$$

10.3 The detection limit is the smallest measurable amount of lead which is distinguishable from the noise of the instrument. It is defined as three standard deviations of a low lead sample which has had seven replicate measurements. After calibration has been verified a low lead sample will be run with seven replicates to calculate the detection limit.

Appendix

Quality Control Materials for Blood Lead Determination

1. New York State Department of Health, Wadsworth Center Lead Poisoning Laboratory, Empire State Plaza, Albany, NY (518) 474-4484.
   Lyophilized reference materials are prepared by dosing animals (goats and cows) with lead acetate. Blood is drawn, aliquotted into amber vials and freeze-dried. Target values are established by calculating the all-method mean of 8-10 reference laboratories. These materials are available to other laboratories.

2. National Institute of Standards and Technology, (NIST) Bldg 202, Room 204, Gaithersburg, MD 20899. (301)975-6776

References

1. Millar, DT, Paschal, DC, Gunter, EW, Stroud, PE, and Lo, J. Determination of blood Lead with Electrothermal Atomic Absorption Using a L'vov Platform and Matrix Modifier. Analyst. 1987; 112; 1701-1704.

2. Perkin Elmer Users Manual; Model 5100 PC Atomic Absorption Spectrophotometer. 1990. Chapters 1-3.

3. New York State Department of Health; Blood Lead Determination by Electrothermal Atomization Atomic Absorption Spectrometry. March 5, 1991.